

## Assessment of chronic exposure to cigarette smoke and its change during pregnancy by segmental analysis of maternal hair nicotine

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This study aimed to investigate the association between biomarkers of fetal exposure to smoking during the whole pregnancy, nicotine in maternal and newborns hair samples, and quantitative measurement of smoking intake and exposure evaluated by maternal self-reported questionnaire. Study subjects were 150 mothers and their newborns from a hospital in Barcelona. A questionnaire including smoking habits was completed in the third trimester of pregnancy and on the day of delivery. Nicotine content was measured in two subsequent segments of maternal hair accounting for the first and last months of pregnancy, and in fetal hair. The geometric mean of nicotine concentration in maternal hair discriminated between nonexposure (3.84 and 2.80 ng/mg in distal and proximal hair segment, respectively) and exposure to cigarette smoke during pregnancy (6.06 and 4.30 ng/mg in distal and proximal hair segment, respectively) ( $P < 0.05$ ), and between these two classes and active smoking (14.40 and 11.08 ng/mg in distal and proximal hair segment, respectively). Maternal hair nicotine was able to differentiate levels of exposure to tobacco smoke and levels of intake. Nicotine concentration in hair from newborns did not differentiate between exposure and nonexposure to environmental tobacco smoke (ETS) in nonsmoking mothers. Finally, chronic exposure to cigarette smoke during pregnancy, assessed by maternal hair nicotine, correlated negatively with anthropometric parameters of newborns.

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### Introduction

The accurate assessment of fetal exposure to smoking through the objective measure of a biomarker could be of major importance for the investigation of the effects of pre- and postnatal environmental exposures to pollutants, including tobacco smoke, in the inception of respiratory diseases (Hanrahan et al., 1992; Nafstad et al., 1996, 1997; Corbo et al., 1996; Lodrup Carlsen et al., 1997; Cook and Strachan, 1998; Strachan and Cook, 1998). Recently, we showed that cord serum cotinine resulted as the most adequate biomarker of fetal exposure to smoking at the end of pregnancy, distinguishing not only active smoking from passive smoking, but also exposure to environmental tobacco smoke (ETS) from nonexposure (Pichini et al., 2000). Nonetheless, the use of a biomarker of acute exposition at delivery would certainly

underestimate smoking consumption or exposition earlier in pregnancy.

In recent years, hair nicotine has been used as a biomarker for assessing chronic exposure to ETS and active smoke, as it can cover wide time-windows (Nafstad et al. 1995; Jaakkola and Jaakkola, 1997; Jaakkola et al., 2001; Segura et al., 2000). Determination of nicotine in the hair of pregnant women proved useful to obtain information on exposure in the whole gestation. In addition, it appeared that newborn hair nicotine could account for all periods of fetal exposure (Eliopoulos et al., 1994, 1996a, b). However, these studies did not attempt to quantify different levels of nicotine intake or exposure to ETS nor evidenced eventual changes in smoking habits during pregnancy performing analyses of different hair sections corresponding to different periods of gestation.

Within the framework of a cohort study on the effects of pre- and postnatal environmental exposures in the inception of atopy and asthma (Asthma Multicenter Infant Cohort Study, AMICS) (Atkinson et al., 1999), we measured biomarkers of acute exposure to tobacco smoke (cotinine in cord serum and maternal and newborn urine) in relation to maternal self-reported questionnaire (Pichini et al., 2000). In

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order to extend the information on smoking intake and exposure to the entire pregnancy, we measured nicotine in maternal and newborn hair and investigated the relation between these biomarkers of chronic exposure and self-reported smoking habits. We further estimated the eventual association between hair nicotine in mothers and newborns and anthropometric parameters of the newborn.

## Materials and Methods

### Subjects

Mothers and their newborns were recruited for the AMICS study. Pregnant women ( $n = 638$ , median age = 29 years) attending the Hospital del Mar in Barcelona during 1997–1998 were invited to participate if they anticipated living in the city during the forthcoming years and had a telephone. A total of 573 pregnant women accepted to be enrolled in the study and gave informed consent. Both parents signed informed consent for their newborns.

From this population, a total of 404 (70.5%) samples of cord serum, 226 (39.4%) maternal urines samples, and 164 (29.0%) newborns' urine samples were collected for the previous study on biomarkers of acute exposure to cigarette smoke (Pichini et al., 2000).

The eligibility criteria for the collection of hair samples were only singleton births, possibility of having matched maternal and newborn hair, maternal hair without any cosmetic treatment that could interfere with nicotine accumulation in hair (Pichini et al., 1997), and a strand length of at least 5 cm. Initially, 322 (56.2%) women met the eligibility criteria for hair collection. Subsequently, when matching these hair samples with those of newborns with a sufficient amount ( $> 1.5$  mg) of hair, the number of samples were reduced to 220. To optimize the power and the cost of the study (the cost of hair nicotine analysis being a limiting economic factor) it was decided *a priori* to include a minimum quantity of mother–newborn pairs, which could allow the statistical power ( $\alpha = 0.05$ ,  $\beta = 0.80$ ) for assessing differences of 10% in hair nicotine concentration by group with an expected standard deviation of 15 ng/mg. These criteria led to the hair analysis of 150 mother–child pair hair samples (50 for each group among nonexposed non smokers, exposed nonsmokers and smokers). No differences in maternal age, smoking habits, and social class (based on occupation) were found between included and excluded participants.

General information on delivery (e.g., birth weight, length, etc.) was recorded from hospital files.

### Questionnaire Information

An exhaustive questionnaire including smoking habits was completed at the first antenatal care visit to the hospital, usually during the third trimester of pregnancy. Mothers were asked if they were nonsmokers, occasional smokers, or daily smokers. If they were daily smokers, they were asked

the average number of cigarettes currently smoked per day and the brand of cigarette. None of the smoker mothers reported smoking on less than daily basis (occasional smokers) or consumption of cigars. Women who reported cessation of smoking at the time of interview (20%) were considered nonsmokers. Regarding exposure to ETS, nonsmoking mothers were asked if they were regularly exposed to ETS, where and by whom (husband or other people in the family or/and at work), the average number of cigarettes and the brand of cigarette smoked by these people, and the average hours of exposure. Tobacco consumption and exposure to ETS were calculated as milligrams of nicotine daily intake (NDI) and mg daily exposure to nicotine (DEN), respectively. In the case of smoker mothers, NDI was obtained from the average number of cigarettes smoked per day multiplied by nicotine content (in mg) of each cigarette (Rosa et al., 1992). In the case of nonsmoking mothers with a passive exposure, DEN was calculated as NDI of the active smoker in the environment of the mother multiplied by the hours during which exposure was reported to occur (as a fraction of 24 h) (Pichini et al., 2000; Segura et al., 2000). If nonsmoking mothers declared contact with more than one smoker, the different exposures were added. A shorter version of the AMICS questionnaire (one-page only) was administered on the day of delivery as a confirmation of the previous one. In a 3.3% of study population ( $n = 5$ ), there was disagreement between the two questionnaires and the second one was considered to be the most reliable (Pichini et al., 2000).

**Hair nicotine** Hair samples were collected on the day of delivery from mothers and their newborns. All the subjects were Caucasian with naturally colored hair. From each subject, a strand of hair of about 1 cm width was collected from the posterior vertex region of the head; the hair was cut at the scalp; and the full length was collected. Hair was cut with scissors swabbed with methanol before every cut. Hair was handled with tweezers and stored in paper envelopes in a smoke-free ambient.

Considering that hair growth is irregular and varies from 0.7 cm/month to a maximum of 1 cm/month (Sachs, 1995), that only a minority of participating women had more than 5–6 cm hair length, and that the object of the study was only the gestation period, a 5 cm hair segment was chosen for nicotine analysis. This strand was divided into two subsequent 2.5 cm segments, starting from hair scalp. The first hair segment (0–2.5 cm segment) accounted to last for 3–4 months of pregnancy and its nicotine content could be compared with that in fetal hair, growing in this gestation period (Larsen, 1997). The second hair segment (2.5–5 cm segment) corresponded to first 3–4 months of pregnancy. Nicotine in hair was measured by gas chromatography coupled to nitrogen–phosphorus detector after extended washing, alkaline digestion of keratin matrix, and solvent extraction of nicotine contained in the matrix (Pascual et al.,

unpublished data). The quantification limit (calculated at <20% error in precision and accuracy) was 0.1 ng nicotine/mg hair when the sample was at least 5 mg. Values under the quantification limit, even if above the detection limit, were not reported because of the uncertainty in the estimated concentrations.

**Statistical Methods** Comparisons of sociodemographic and clinical data of mothers and newborns in terms of self-reported smoking status were performed by  $\chi^2$  tests for categorical variables and ANOVA statistics for continuous variables. Since hair nicotine values did not follow a Normal distribution (distribution skewed to the right), nicotine concentrations were log transformed in order to fit a Normal distribution. In order to assess a dose–response relation between smoking habits and hair nicotine levels, two different categories of exposure by DEN and active smoking by NDI were defined. Furthermore, to compare nicotine concentrations among nonexposed nonsmokers, exposed nonsmokers, and smokers adjusting for potential confounders, such as maternal age, child's sex, order in family and gestational age, a multiple linear regression analysis was conducted. The same process was used to relate the mean nicotine amounts in the two subsequent maternal hair segments and neonatal somatometric parameters. The analyzed parameters were: birth weight, length, head circumference, and two measures of infant body proportionality: ponderal index (PI) and brain:body ratio (BBR) (Lindley et al., 2000a). PI is defined as:  $\text{birth weight (g)}/\text{length (cm)}^3 \times 100$ . A lower PI indicates a longer, thinner infant whereas a higher PI indicates a shorter and/or fatter infant. Typical values of PI are between 2.6 and 2.9. BBR is an indicator of head-to-body proportionality and it is calculated as:  $100 \times [0.037 \times \text{head circumference (cm)}^{2.57}]/\text{birth weight (g)}$ . A higher BBR indicates a higher proportion of birth weight residing in the brain, being values for full-term infants around 9–10% (Lindley et al., 2000a). All analyses were performed using Stata, version 5.0 (StataCorp, 1997, College Station, TX, USA).

## Results

Table 1 describes the sociodemographic characteristics and neonatal features of the study population. Age was higher among exposed nonsmoker women, while nonexposed nonsmokers had their first child in a higher proportion. Some of the anthropometric measures of newborns appeared to be related with smoking (i.e., weight and length were lower in smokers).

Distribution of nicotine levels in hair from mothers and newborns are reported in Table 2 as percentiles. It can be noted that in the case of maternal hair, nicotine could practically be quantified in all samples, while in the case of newborn hair, nicotine could not be quantified in 30.7% of samples (Table 3).

The median level of nicotine in both proximal (0–2.5 cm) and distal (2.5–5 cm) segment of hair from smoker mothers

was more than two-fold higher than that of exposed nonsmokers and at least three times higher than that of nonexposed nonsmokers (Table 3). In addition, an increasing trend of median level of hair nicotine was observed in the two groups of milligrams of DEN in exposed nonsmokers and milligrams of NDI in smoker mothers. Results were confirmed when considering geometric means of hair nicotine in the three groups of mothers, adjusting for confounding factors. Indeed, the geometric mean of nicotine concentration both in proximal and distal segment of maternal hair was statistically different between non-exposed and exposed nonsmokers, and between these two classes and smokers, and furthermore was statistically different between two levels of exposure to tobacco smoke and levels of intake.

Furthermore, it can be observed in Table 3 and in Fig. 1 that hair nicotine in the three groups of mothers was significantly higher in the first months of pregnancy.

Nonetheless, nicotine concentration in the proximal segment of mothers' hair well correlated with that of the distal segment ( $r=0.92$ ). Additionally, maternal hair nicotine in both proximal and distal segments presented a good correlation with all the biomarkers of smoking habits at the end of pregnancy that were estimated in a previous study (Pichini et al., 2000) ( $r=0.70$  and  $0.72$  with cord serum cotinine;  $r=0.74$  and  $0.79$  with maternal urinary cotinine; and  $r=0.70$  and  $0.69$  with newborn urinary cotinine, respectively). Conversely, no correlation was found between maternal hair nicotine and newborn hair nicotine, neither between newborn hair nicotine and cord blood or urinary cotinine (all  $r<0.05$ ).

Indeed, nicotine concentration in neonatal hair of newborns from nonexposed nonsmokers and exposed nonsmokers was similar (Table 4). Only newborns from smoking mothers showed a lower proportion in the "not quantifiable" hair nicotine concentration and a higher proportion in the upper range of hair nicotine.

The relation between somatometric measures of the newborn and smoking habit, assessed by hair nicotine is illustrated in Table 5. Hair nicotine was calculated as the mean of the values encountered in the proximal and distal segments of maternal hair and then divided into three exposure categories: low (<3 ng/mg, reference category), medium (between 3 and 18 ng/mg), and high (>18 ng/mg). Mean values (and standard deviation) of neonatal variables were reported for the reference category, whereas for the other two categories, changes according to reference category were described. A statistical decrease in birth weight and head circumference of the newborn was observed for the high exposure category, while PI and BBR did not show statistical changes with respect to maternal smoking habit. This finding indicates that even if maternal heavy active smoking is associated with lower neonatal somatometric indexes, no body disproportionality was evidenced in newborns from heavy-smoker mothers.

**Table 1.** Characteristics of the study population according to self-reported smoking habits.

	Nonexposed-nonsmokers		Exposed-nonsmokers		Smokers	
	No.	% or mean (SE)	No.	% or mean (SE)	No.	% or mean (SE)
Total		48		51		51
Mothers						
Age (Mean (SE))	48	28.5 (5.6)	51	30.9* (4.6)	51	28.3 (5.0)
<i>Father's social class (%)</i>						
Professional	2	5.1	1	2.2	1	2.3
Managerial and technical	6	15.4	7	15.6	3	7.0
Skilled (nonmanual)	5	12.8	13	28.9	9	20.9
Skilled (manual)	16	41.0	18	40.0	20	46.5
Partly skilled	8	20.5	6	13.3	9	20.9
Unskilled	2	5.1	0	0	1	2.3
<i>Child</i>						
Male (%)	25	52.1	28	54.9	29	56.9
Female (%)	23	47.9	23	45.1	22	43.1
<i>Order in family (%)</i>						
1	30	65.2	21	44.7	21	46.7
2	10	21.7	17	36.2	20	44.4
> 2	6	13.0	9	19.2	4	8.9
Birth weight (g) (Mean (SE))	48	3271 (500.6)	50	3306 (461.4)	51	3109 (398.4)
Length (cm) (mean (SE))	45	49.6 (2.7)	48	49.6 (2.5)	48	48.9 (1.7)
Cranial perimeter in (cm) (Mean (SE))	45	34.3 (1.7)	48	34.7 (1.3)	48	34.1 (1.6)
Ponderal index (g/cm <sup>3</sup> ) (Mean (SE))	45	2.7 (0.3)	48	2.7 (0.3)	49	2.7 (0.3)
Brain:body weight ratio (cm <sup>2.57</sup> /g) (mean (SE))	45	10.2 (1.6)	48	10.4 (1.9)	48	10.5 (1.2)
Small for gestational age	3	6.3	9	18.0	8	15.7
Low weight (<2500 g) (%)	2	4.1	2	3.9	2	3.9
Prematurity (<37 weeks) (%)	4	8.3	5	10.0	2	3.9

\*P-Value <0.05 in relation to 'Nonexposed-nonsmokers'.

**Table 2.** Distribution of hair nicotine and urinary and cord blood cotinine in the study population.

	NQ		Percentile						
	No.	(%)	Min	5th	25th	50th	75th	95 <sup>th</sup>	Max
Mother hair nicotine (0–2.5 cm) (ng/mg)	150	0	0.55	1.06	2.37	5.01	10.00	39.17	99.12
Mother hair nicotine (2.5–5 cm) (ng/mg)	150	1.3	0.50	1.34	3.37	6.45	14.10	54.08	169.60
Newborn hair nicotine (ng/mg)	150	30.7	0.33	0.60	1.20	2.08	4.47	9.15	14.80

## Discussion

Results from this study suggest that measurement of nicotine in maternal hair was useful in predicting cumulative exposure during the entire pregnancy. Indeed, active and passive smoking, classified as NDI and DEN, together with nonexposure to ETS could be differentiated by mother hair nicotine. As previously reported (Pichini et al., 2000), these two measures, even suffering from some drawbacks (NDI

does not account for intersubject variability in uptake, metabolism, and elimination of tobacco smoke products; DEN does not take into consideration air flow, ventilation, proximity to active smokers) may be seen as an attempt to measure objectively source strength.

The population selected for this investigation did not show any difference in demographic or socioeconomic characteristics with excluded women, respecting the socio-demographic distribution and smoking habits of the initial

**Table 3.** Association between self-reported smoking habit and maternal hair nicotine.

Self-reported smoking habit	Mother hair nicotine (ng/mg)					
	(0–2.5 cm)			(2.5–5 cm)		
	<i>n</i>	Median	Geom. mean <sup>a</sup>	<i>n</i>	Median	Geom. mean <sup>a</sup>
Nonexposed-nonsmokers	48	3.02	2.80	47	3.75	3.84
<i>Exposed-nonsmokers</i>	51	4.07	4.30*	50	5.44	6.06*
DEN <sup>b</sup>						
< 2	19	2.69	2.96	18	3.71	3.59
≥ 2	31	6.40	5.41**	31	7.36	7.58**
<i>Smokers</i>	51	10.00	11.08****	51	14.71	14.40****
NDI <sup>c</sup>						
< 4.9	25	6.27	7.26	25	10.57	8.96
≥ 4.9	26	14.17	16.63****	26	20.97	22.72****

<sup>a</sup>Adjusted for maternal age, sex of child, order in family and gestational age

<sup>b</sup>DEN: daily exposure to nicotine = (as mg nicotine) =  $\sum_{\text{smokers}} [\text{NDI}_{\text{smokers}} (\text{hours spent with the smoker}/24 \text{ h})]$ .

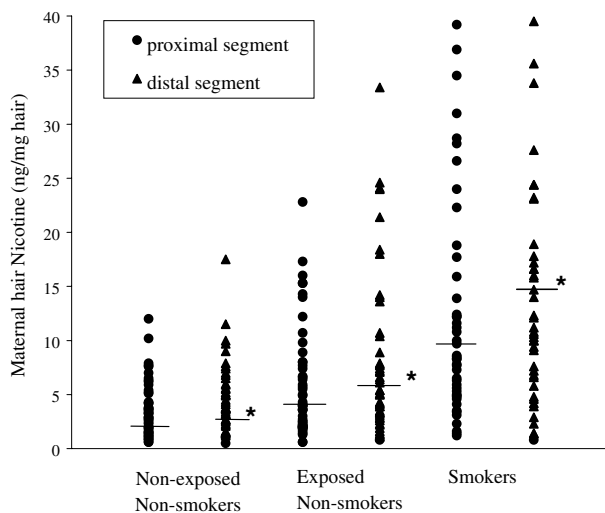
<sup>c</sup>NDI: Nicotine daily intake (as mg nicotine = (mg nicotine/cigarette) (number of smoked cigarettes/day).

\* $P < 0.05$  in relation to 'nonexposed-nonsmokers'.

\*\*\* $P < 0.05$  in relation to 'exposed-nonsmokers'.

\*\* $P < 0.05$  in relation to first group of DEN.

\*\*\*\* $P < 0.05$  in relation to first group of NDI.



**Fig. 1.** Nicotine concentration in the proximal and distal segment of hair strand in the three groups of mothers. \* $P < 0.05$  in relation to proximal hair segment.

study population, in which biomarkers of acute exposure to cigarette smoke were investigated (Pichini et al., 2000).

In any case, even in this restricted study population, hair nicotine accurately assessed smoking habit and evidenced through segmental hair analysis that, as expected, passive and active smoking were higher in the first months of pregnancy.

For the first time, measurement of nicotine was executed in two subsequent segments of maternal hair, accounting for the

**Table 4.** Association between self-reported smoking habit and new-born hair nicotine in tertiles.

Self-reported smoking habit	New-born hair nicotine in tertiles (ng/mg)		
	<i>n</i> (%)		
	NQ	0.3–2.1	> 2.1
<i>Nonexposed-nonsmokers</i>	19 (39.6)	16 (33.3)	13 (27.1)
<i>Exposed-nonsmokers</i>	18 (35.3)	20 (39.2)	13 (25.5)
DEN <sup>a</sup>			
< 2	7 (36.8)	10 (52.6)	2 (10.5)
≥ 2	11 (35.5)	10 (32.3)	10 (32.3)
<i>Smokers</i>	9 (17.7)**	17 (33.3)	25 (49.0)***
NDI <sup>b</sup>			
< 4.9	5 (20.0)	9 (36.0)	11 (44.0)
≥ 4.9	4 (15.4)	8 (30.8)	14 (53.9)

NQ: not quantifiable.

<sup>a</sup>DEN: daily exposure to nicotine (as mg nicotine) =  $\sum_{\text{smokers}} [\text{NDI}_{\text{smokers}} (\text{hours spent with the smoker}/24 \text{ h})]$

<sup>b</sup>NDI: Nicotine daily intake (as mg nicotine) = (mg nicotine/cigarette) (number of smoked cigarettes/day)

\* $P < 0.05$  in relation to 'nonexposed-nonsmokers'.

\*\*\* $P < 0.05$  in relation to 'exposed-nonsmokers'.

first and the last months of pregnancy. In particular, it was decided to take a 5 cm portion of the hair strand and divide it into two 2.5 cm segments. Likely, this hair portion did not exactly cover the whole pregnancy for the entire study population, but it was considered the best compromise

**Table 5.** Changes (mean and SD) in reproductive variables as a function of nicotine measured in the maternal hair.

Hair nicotine (ng/mg hair)	No. (%)	Birth weight (g) <sup>a</sup>	Length (cm) <sup>b</sup>	Head circumference (cm) <sup>b</sup>	PI (g/cm <sup>3</sup> ) <sup>c</sup>	BBR (cm <sup>2.57</sup> /g) <sup>a</sup>
< 3 (reference category)	35 (23.65)	3292.32 (74.13)	48.78 (0.42)	34.34 (0.29)	2.827 (0.060)	10.301 (0.247)
3 to 18	90 (60.81)	-74.71 (87.54)	0.12 (0.44)	-0.20 (0.30)	-0.093 (0.063)	0.156 (0.293)
≥ 18	23 (15.54)	-247.07 (118.64)*	-0.70 (0.60)	-0.83 (0.41) *	-0.113 (0.087)	0.188 (0.395)

\* $P < 0.05$  in relation to first group of mother hair nicotine concentration.

<sup>a</sup>Adjusted for gestational age.

<sup>b</sup>Adjusted for sex of child, and gestational age.

<sup>c</sup>Adjusted for sex of child.

PI: ponderal index =  $100 \times [\text{birth weight (g)} / \text{length (cm)}^3]$ .

BBR—brain:body weight ratio =  $100 \times [0.037 \times \text{cranial perimeter (cm)}^{2.57} / \text{birth weight (g)}]$ .

between characteristics of interpersonal variation of hair growth (Sachs, 1995) and average hair length in participating women. In addition, being conservative in the number of centimeter analyzed, we could be sure to exclude hair grown in the period before pregnancy.

Using this approximation, the distal 2.5 cm hair segment was considered to represent the last 3–4 months of pregnancy, while the proximal 2.5 cm hair segment accounted for the previous 3–4 months, commonly defined as early pregnancy (Larsen, 1997).

It can be underlined that early pregnancy is the period corresponding to embryogenesis, and thus higher exposure to tobacco smoke constituents at this time of gestation could be relevant with regard to a correct neonatal neurodevelopment (Larsen, 1997).

As reported by other authors, a retrospective questionnaire can suffer problems of random and systematic error in the exposure assessment (Jaakkola et al., 2001). Indeed, both the fact that nicotine concentration was statistically higher in the distal segment of maternal hair and that hair nicotine levels found in this study population were generally higher than those observed in other studies (Eliopolus et al., 1994; Nafstad et al. 1998; Jaakkola et al., 2001) supports both maternal unawareness and underestimation of ETS exposure, as well as an under-reporting or an unwillingness to declare real active smoking during the whole pregnancy (Pichini et al., 2001). High concentrations of hair nicotine in nonsmoker women are in agreement with high levels of cord serum and urinary cotinine found in the same individuals (Pichini et al., 2000).

Finally, chronic exposure to smoking, reported as maternal hair nicotine, correlates to acute exposure measured by cord serum cotinine and maternal and newborn urinary cotinine (Pichini et al., 2000). In this concern, owing to the good correlation between maternal hair nicotine and cord serum cotinine, one could support the use of cord serum cotinine as a biomarker of smoking habit not only at the end of pregnancy but also during the whole gestation, taking into consideration the high cost and complex procedure of hair analysis in comparison with the simple and high throughput

immunoanalysis of serum cotinine. However, we believe that even if cord serum cotinine was shown to fairly generally reflect a chronic exposure, this could not be true in clinical settings where the entire fetal exposure of each particular case is of interest for the diagnosis of respiratory diseases in childhood.

Results from our study show that maternal hair nicotine even in a number of mothers fewer than those analyzed in the case of cord serum cotinine (150 vs. 404 subjects) and with a shorter range of concentrations (0.5–169.6 ng/mg vs 0.2–910 ng/ml) is able to distinguish a hundred-fold exposure differential between smokers and nonsmokers, to differentiate levels of exposure to ETS and exposure-related neonatal effects, appearing to be the “clinical gold standard” of chronic exposure to cigarette smoking during pregnancy.

There is another potential source of misclassification of exposure since it is not possible to detect clearly as to which proportion of nicotine is directly deposited on hair from environment or comes from active or passive inhaled smoke. A great portion of nicotine deposited on the exterior of hair can be eliminated through a preliminary washing, but it has been shown that even an extended procedure cannot eliminate completely external contamination (Kidwell and Blank, 1995). Nonetheless, the problems of overlapping levels for different categories of exposure (e.g., smokers of less than 10 cigarettes and high exposed nonsmokers) is a common problem for all biological markers of exposure to smoke and in general to xenobiotics (Jaakkola and Jaakkola, 1997). In any case, the point is that the real interest is not to detect accurately a smoker or a nonsmoker, but the measurement of an overall exposure to smoking, which is the determinant for health consequences on newborns.

Unfortunately, newborn hair nicotine did not appear as a valuable predictor of different levels of fetal exposure to cigarette smoke. Neonatal hair nicotine could only discriminate active smoking by the mother, but it did not show any association with chronic exposure and nonexposure to ETS as reported by the questionnaire, nor it correlated with the maternal hair nicotine and biomarkers of acute exposure to smoking.

Since all the newborns enrolled in this study did not present any particular pathology of clinical relevance, it can be discarded that transplacental passage or blood irrigation could influence accumulation of nicotine together with the other tobacco smoke constituents by fetus. Conversely, it can be hypothesized that variability in hair nicotine concentration of newborns presenting a similar exposure to cigarette smoking can be because of the differences in the fetal physiology regarding the formation, development, growth, and melanin content of fetal hair (Pötsch et al., 1997). However, the real factors that can explain fetal accumulation of cigarette smoke products are still unknown.

As extensively reported by international literature (Hogue et al., 1987; Jaakkola et al., 2001), maternal active and passive smoking seriously affects fetal health. Results from this study demonstrated that maternal tobacco consumption during pregnancy is inversely associated with classical neonatal somatometric measures such as birth weight and head circumference. However, even presenting intrauterine growth retardation, newborns from smoking mothers showed a body proportionality (PI and BBR) similar to that of infants from nonsmoking mothers and hence a symmetric growth retardation. A similar finding was also reported in the case of acute exposure to smoking, measured by cord serum cotinine (Pichini et al., 2002). It has been suggested that infants symmetrical in their growth retardation present a higher incidence of neonatal complications and hospitalization during the first year of life with respect to asymmetrical newborns with long term consequences for subsequent infant morbidity (Vik et al., 1996; Lindley et al., 2000b).

Some authors demonstrated that also maternal exposure to ETS during pregnancy, assessed by hair nicotine in maternal hair, could impair the length of gestation and fetal growth (Jaakkola et al., 2001). In our study, we could not observe any adverse effect of maternal exposure to ETS when compared to nonexposure in nonsmoking mothers. However, our statistical power was lower.

Furthermore, the reference category of nonexposure to ETS, reported in the aforementioned study as hair nicotine <0.75 ng/mg hair, was practically absent in our population. In fact, even in women who declared a nonexposure, levels of maternal hair nicotine were consistent with an unconscious exposure to ETS.

In conclusion, maternal hair nicotine appeared to be an adequate biomarker of chronic exposure to cigarette smoking during pregnancy because of its ability to be associated with levels of active smoking, level of passive maternal exposure to ETS, and to neonatal effects such as birth weight and head circumference. In agreement with other authors (Vik et al., 1996; Jaakkola et al., 2001), these somatometric effects, together with the risk of small-for-gestational-age and preterm delivery, were associated with the exposure in the last trimester of pregnancy.

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